

SEQUENCE LISTING

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<120> TAQ DNA POLYMERASES HAVING AN AMINO ACID SUBSTITUTION AT E681 AND
HOMOLOGS THEREOF EXHIBITING IMPROVED SALT TOLERANCE

<130> PB9944

<140> US 10/049,358
<141> 2002-05-17

<150> PCT/US00/22150
<151> 2000-08-10

<150> US 60/154,739
<151> 1999-09-17

<150> US 60/150,167
<151> 1999-08-21

<160> 16

<170> PatentIn version 3.3

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<212> PRT
<213> Thermus aquaticus

<400> 1

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Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
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Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
 115 120 125
 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
 130 135 140
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
 145 150 155 160
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
 165 170 175
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
 180 185 190
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
 260 265 270
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
 275 280 285
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn

370

375

380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735
 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750
 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765
 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780
 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800
 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
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 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

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 <213> Thermus aquaticus

<400> 2

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 20 25 30

Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala
 35 40 45
 Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala
 50 55 60
 Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu
 65 70 75 80
 Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu
 85 90 95
 Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser
 100 105 110
 Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr
 115 120 125
 Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn
 130 135 140
 Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg
 145 150 155 160
 Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr
 165 170 175
 Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val
 180 185 190
 Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly
 195 200 205
 His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe
 210 215 220
 Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys
 225 230 235 240
 Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro
 245 250 255
 Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser
 260 265 270
 Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg
 275 280 285
 Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser

290

295

300

Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly
305 310 315 320

Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val
325 330 335

Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser
340 345 350

Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His
355 360 365

Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp
370 375 380

Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Tyr Gly Val Leu Tyr
385 390 395 400

Gly Met Ser Ala His Arg Leu Ser Gln Arg Leu Ala Ile Pro Tyr Glu
405 410 415

Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val
420 425 430

Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr
435 440 445

Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala
450 455 460

Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met
465 470 475 480

Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys
485 490 495

Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val
500 505 510

His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val
515 520 525

Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val
530 535 540

Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys
545 550 555 560

Glu

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<212> PRT
<213> *Thermus aquaticus*
<400> 3

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Val Ala Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
20 25 30
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60
Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
115 120 125
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
130 135 140
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
165 170 175
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
180 185 190
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
 260 265 270

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
 275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His

465	470	475	480
Pro Phe Asn Leu	Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp		
	485	490	495
Glu Leu Gly Leu	Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg		
	500	505	510
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile			
	515	520	525
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr			
	530	535	540
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu			
	545	550	555
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser			
	565	570	575
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln			
	580	585	590
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala			
	595	600	605
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly			
	610	615	620
Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr			
	625	630	635
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro			
	645	650	655
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Tyr Gly Val Leu Tyr Gly			
	660	665	670
Met Ser Ala His Arg Leu Ser Gln Arg Leu Ala Ile Pro Tyr Glu Glu			
	675	680	685
Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg			
	690	695	700
Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val			
	705	710	715
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg			
	725	730	735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
740 745 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
820 825 830

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<212> DNA
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acaaacatac gagccggaag cataaagtgt aaagcctggg gtgcctaattg agtgagctaa

120

ctcacattaa attgcgttgc gctcactgcc cgctttccag tcgggaaacc tgtcgtgcc

180

gctgcattaa tgaaatcggc caacgcgcgg ggagaggcgg tttgcgtatt gggcgccagg	240
gtgggtttttc ttttcaccag gtgagacggg caacagctga ttgcccttca ccgcctggcc	300
ctgagagagt tgcagcaagc ggtccacg	328

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cacaaacata cgagccggaa gcataaagtg taaagcctgg ggtgcctaata gagtgagcta	120
actcacatit aattgcgttg cgctcactgc ccgctttcca gtcgggaaac ctgtcgtgcc	180
agctgcatta atggaatcgg ccaacgcgcg gggagaggcg gtttgcttat tgggcgccag	240
ggtaggttttt cttttcacca agtgagacgg gcaacagctg attgcccttc accgcctggc	300
cctgagagag ttgcagcaag cgggtcca	327

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caagcccaca aacaacacgc gcgctccaac caaaattcct ccaccacccc taccacacca 120
ccaanaancc annnnnnnnn nnnnnnnnnn nnnnnnnnath cnnncnnnn nnnnnnnnnn 180
nnnnnnnnnn 189

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<213> Unknown

<220>
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aacaaccacg gcgctccaa ccaacctaat tccctccacc caccctccca caccacccac 120

cacccaccac ccaaccaccc accaccgatc cacaaatcca cccacccccac accacaccac 180
 caccaacgaa aaacaagacc a 201

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 <213> Artificial Sequence

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<210> 11
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 <212> DNA
 <213> Artificial Sequence

<220>
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<400> 11
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<210> 12
 <211> 20
 <212> DNA
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<220>
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 gt 62

<210> 14
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gtc 63

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